any of the well-known methods for converting triglycerides to free fatty acids or esters of fatty acids including base hydrolysis, acid hydrolysis, or enzymatic hydrolysis. The hydrolysis should be carried out at as low a temperature as possible (e.g., room temperature to 60°C) and under nitrogen to minimize breakdown of the omega-3 HUFAs. After hydrolysis is completed, the nonsaponifiable compounds are extracted into a solvent such as ether, hexane or chloroform and removed. The remaining solution is then acidified by addition of an acid such as HCl, and the free fatty acids extracted into a solvent such as hexane, ether, or chloroform. The solvent solution containing the free fatty acids can then be cooled to a temperature low enough for the non-HUFAs to crystallize, but not so low that HUFAs crystallize. Typically, the solution is cooled to between about -60°C and about -74°C. The crystallized fatty acids (saturated fatty acids, and mono-, di-, and tri-enoic fatty acids) can then be removed (while keeping the solution cooled) by filtration, centrifugation or settling. The HUFAs remain dissolved in the filtrate (or supernatant). The solvent in the filtrate (or supernatant) can then be removed leaving a mixture of fatty acids which are >90% purity in either omega-3 HUFAs or HUFAs which are greater than or equal to 20 carbons in length. The purified omega-3 highly unsaturated fatty acids can then be used as a nutritional supplement for humans, as a food additive, or for pharmaceutical applications. For these uses the purified fatty acids can be encapsulated or used directly. Antioxidants can be added to the fatty acids to improve their stability.

The advantage of this process is that it is not necessary to go through the urea complex process or other expensive extraction methods, such as supercritical CO_2 extraction or high performance liquid chromatography, to remove saturated and mono-unsaturated fatty acids prior to cold crystallization. This

advantage is enabled by starting the purification process with an oil consisting of a simple fatty acid profile such as that produced by Thraustochytrids (3 or 4 saturated or monounsaturated fatty acids with 3 or 4 HUFAs, two groups of fatty acids widely separated in terms of their crystallization temperatures) rather than a complex oil such as fish oil with up to 20 fatty acids (representing a continuous range of saturated, mono-, di-, tri-, and polyenoic fatty acids, and as such, a series of overlapping crystallization temperatures).

In a preferred process, the omega-3 HUFA enriched oils can be produced through cultivation of strains of the genus Thraustochytrium. After the oils are extracted from the cells by any of several well-known methods, the remaining extracted (lipids removed) biomass which is comprised mainly of proteins and carbohydrates, can be sterilized and returned to the fermenter, where the strains of Thraustochytrium can directly recycle it as a nutrient source (source of carbon and nitrogen). No prehydrolysis or predigestion of the cellular biomass is necessary. Extracted biomass of the genus Schizochytrium can be recycled in a similar manner if it is first digested by an acid and/or enzymatic treatment.

As discussed in detail above, the whole-cell biomass can be used directly as a food additive to enhance the omega-3 highly unsaturated fatty acid content and nutritional value of processed foods for human intake or for animal feed. When used as animal feed, omega-3 HUFAs are incorporated into the flesh or other products of animals. The complex lipids containing these fatty acids can also be extracted from the whole-cell product with solvents and utilized in a more concentrated form (e.g., encapsulated) for pharmaceutical or nutritional purposes and industrial applications. A further aspect of the present invention includes introducing omega-3 HUFAs from the foreoging sources into humans for the treatment of various

diseases. As defined herein, "treat" means both the remedial and preventative practice of medicine. The dietary value of omega-3 HUFAs is widely recognized in the literature, and intake of omega-3 HUFAs produced in accordance with the present invention by humans is effective for treating cardiovascular diseases, inflammatory and/or immunological diseases and cancer.

The present invention will be described in more detail by way of working examples. Species meeting the selection criteria described above have not been described in the prior art. By employing these selection criteria, the inventor isolated over 25 potentially promising strains from approximately 1000 samples screened. Out of the approximate 20,500 strains in the American Type Culture Collection (ATCC), 10 strains were later identified as belonging to the same taxonomic group as the strains isolated by the inventor. Those strains still viable in the Collection were procured and used to compare with strains isolated and cultured by the disclosed procedures. The results of this comparison are presented in Examples 5 and 6 below.

Since the filing of the parent case, recent developments have resulted in revision of the taxonomy of the Thraustochytrids. The most recent taxonomic theorists place them with the algae. However, because of the continued taxonomic uncertainty, it would be best for the purposes of the present invention to consider the strains as Thraustochydrids (Order: Thraustochytriales; Family: Thraustochytriaceae; Genus: Thraustochytrium or Schizochytrium). The most recent taxonomic changes are summarized below.

All of the strains of unicellular microorganisms disclosed and claimed herein are members of the order Thraustochytriales. Thraustochytrids are marine eukaryotes with a rocky taxonomic history. Problems with the taxonomic placement of the Thraustochytrids have been reviewed most recent by Moss (1986), Bahnweb and Jackle (1986) and Chamberlain and Moss (1988). For

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convenience purposes, the Thraustochytrids were first placed by taxonomists with other colorless zoosporic eukaryotes in the Phycomycetes (algae-like fungi). The name Phycomycetes, however, was eventually dropped from taxonomic status, and the Thraustochytrids retained in the Oomycetes (the biflagellate zoosporic fungi). It was initially assumed that the Oomycetes were related to the heterokont algae, and eventually a wide range of ultrastructural and biochemical studies, summarized by Barr (1983) supported this assumption. The Oomycetes were in fact accepted by Leedale (1974) and other phycologists as part of the heterokont algae. However, as a matter of convenience resulting from their heterotrophic nature, the Oomycetes and Thraustochytrids have been largely studied by mycologists (scientists who study fungi) rather than phycologists (scientists who study algae).

From another taxonomic perspective, evolutionary biologists have developed two general schools of thought as to how eukaryotes evolved. One theory proposes an exogenous origin of membrane-bound organelles through a series of endosymbioses (Margulis (1970); e.g., mitochondria were derived from bacterial endosymbionts, chloroplasts from cyanophytes, and flagella from spirochaetes). The other theory suggests a gradual evolution of the membrane-bound organelles from the non-membrane-bounded systems of the prokaryote ancestor via an autogenous process (Cavalier-Smith 1975). Both groups of evolutionary biologists, however, have removed the Oomycetes and ~~thraustochytrids~~ Thraustochytrids from the fungi and place them either with the chromophyte algae in the kingdom Chromophyta (Cavalier-Smith 1981) or with all algae in the kingdom Protoctista (Margulis and Sagan (1985).

With the development of electron microscopy, studies on the ultrastructure of the zoospores of two genera of Thraustochytrids, Thraustochytrium and Schizochytrium, (Perkins 1976; Kazama 1980; Barr 1981)

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have provided good evidence that the Thraustochytriaceae are only distantly related to the Oomycetes. Additionally, more recent genetic data representing a correspondence analysis (a form of multivariate statistics) of 5S ribosomal RNA sequences indicate that Thraustochytriales are clearly a unique group of eukaryotes, completely separate from the fungi, and most closely related to the red and brown algae, and to members of the Oomycetes (Mannella et al. 1987). Recently however, most taxonomists have agreed to remove the Thraustochytrids from the Oomycetes (Bartnicki-Garcia 1988).

In summary, employing the taxonomic system of Cavalier-Smith (1981, 1983), the Thraustochytrids are classified with the chromophyte algae in the kingdom Chromophyta, one of the four plant kingdoms. This places them in a completely different kingdom from the fungi, which are all placed in the kingdom Eufungi. The taxonomic placement of the Thraustochytrids is therefore summarized below:

Kingdom:	Chromophyta
Phylum:	Heterokonta
Order:	Thraustochytriales
Family:	Thraustochytriaceae
Genus:	Thraustochytrium or Schizochytrium

Despite the uncertainty of taxonomic placement within higher classifications of Phylum and Kingdom, the Thraustochytrids remain a distinctive and characteristic grouping whose members remain classifiable within the order Thraustochytriales.

Omega-3 highly unsaturated fatty acids are nutritionally important fatty acids for both humans and animals. Currently the only commercially available source of these fatty acids is from fish oil. However, there are several significant problems with the use of fish oil as a food or feed additive or supplement.

First and most significantly, fish oils have a strong fishy taste and odor, and as such cannot be added to processed foods as a food additive, without negatively affecting the taste of the food product. This is also true for many of its applications as an animal food or feed additive. For example, experiments by the inventor and others have indicated that laying hens readily go off their feed when fed for more than a few days on feed enriched with fish oils. Fish oils are very unstable, easily becoming rancid and thereby decreasing the palatability and nutritional value of feed.

Secondly, fish oils generally only contain 20-30% omega-3 HUFAs. Desirable omega-3 HUFA contents in marine larval fish and shrimp feeds can be as high as 5-10% of their dry weight. To constitute an appropriate synthetic diet containing 5-10% omega-3 HUFAs could require a diet of 15-30% fish oil. Such a synthetic diet would not be the most suitable for these larval organisms either in terms of palatability, digestibility, or stability (Sargent et al. (1989). In terms of human nutrition, the other 70-80% of fatty acids in fish oil are saturated and omega-6 fatty acids, fatty acids which can have deleterious health effects for humans. Processes for the isolation of pure omega-3 fatty acids from fish oils are involved and expensive, resulting in very high prices ($200-$1000/g) for pure forms of these fatty acids, much too expensive for use as a food or feed additive (Sigma Chemical, Co., 1988; CalBiochem Co., 1988).

Third, most feeds currently used by the aquaculture industry are grain based feeds, and as such, are relatively low in omega-3 HUFA content. Recent surveys of seafood products have demonstrated that fish and shrimp produced by aquaculture farms generally only have 1/3-1/2 the omega-3 HUFA content of wild caught fish and shrimp (Pigott 1989). For aquacultured organisms, many which are prized because of their mild, non-fishy taste,

increasing the fish oil content of their food is not effective, because it results in a fish-tasting product.

As a result of the problems described above, there is an important need for development of alternative (non-fish based) sources of omega-3 HUFAs.

The microbial product of the present invention can be used as a food or feed supplement to provide an improved source of omega-3 highly unsaturated fatty acids which has significant advantages over conventional sources. Poultry fed a diet supplemented with the microbial product incorporate the omega-3 highly unsaturated fatty acids into body tissues and into eggs. The eggs exhibit no fishy odor or taste, no change in yolk color. The poultry do not stop eating the supplemented feed, as they do with fish oil-supplemented feed. Feed supplemented with the microbial product of the present invention has a normal shelf life and does not become rancid upon standing at room temperature for several days. The eggs and flesh of poultry fed according to the invention are useful in human nutrition as sources of omega-3 highly unsaturated fatty acids, yet are low in omega-6 fatty acid content and lack a fishy flavor.

The microbial product of the present invention is also of value as a source of omega-3 highly unsaturated fatty acids for fish, shrimp and other products produced by aquaculture. The product can be added directly as a supplement to the feed or it can be fed to brine shrimp or other live feed organisms intended for consumption by the aquacultured product. The use of such supplement enables the fish or shrimp farmer to bring to market an improved product retaining the taste advantages provided by aquaculture but having the high omega-3 highly unsaturated fatty acid content of wild caught fish coupled to the additional health advantage of reduced omega-6 fatty acid content.

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Brief Description of the Figures

Figure 1 is a bar graph showing the effects of various media supplements on fatty acid yield, using Thraustochytrium sp. UT42-2 (ATCC No. 20891), a strain isolated according to the selection method of the invention as a test strain. The experimental procedure is described in Example 2. Ordinate: fatty acid yield, normalized to control, FFM media without supplements. Abscissa: specific additions, 1) 2x "B"-vitamin mix; 2) 2x "A" vitamin mix; 3) 2x PI metals; 4) 28mg/l KH_2PO_4; 5) treatments 2), 3) and 4) combined; and 6) 480mg/l KH_2PO_4.

Figure 2 is a graphical representation of highly unsaturated fatty acid production in newly isolated strains of the invention, represented by ■, and previously isolated strains represented by +. Each point represents a strain, the position of each point is determined by the percent by weight of total fatty acids which were omega-3 highly unsaturated fatty acids (abscissa) and the percent by weight of total fatty acids which were omega-6 fatty acids (ordinate). Only those strains of the invention were plotted wherein less than 10.6% (w/w) of total fatty acids were omega-6 and more than 67% of total fatty acids were omega-3. Data from Table 4.

Figure 3 is a graphical representation of highly unsaturated fatty acid production in newly isolated strains of the invention, represented by ■, and previously isolated strains, represented by +. Each point represents a strain, the position of each point is determined by the percent by weight of total fatty acids which were omega-3 highly unsaturated fatty acids (abscissa) and percent of weight of total fatty acids which were eicosapentaenoic acid (EPA C20:5w3) (ordinate). Only those strains of the invention were plotted wherein more than 67% (w/w) of total fatty acids were omega-3 and more than 7.8% (w/w) of total fatty acids were C20:5w3.

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Figure 4 is a graphical representation of omega-3 highly unsaturated fatty acid composition in newly isolated strains of the invention, represented by □, and previously isolated strains, represented by +. Each point represents a separate strain. Values on the abscissa are weight fraction of total omega-3 highly unsaturated fatty acids which were C20:5w3 and on the ordinate are weight fraction of total omega-3 fatty highly unsaturated acids which were C22:6w3. Only strains of the invention were plotted having either a weight fraction of C20:5w3 28% or greater, or a weight fraction of C22:6w3 greater than 93.6%.

Figure 5 is a graph showing growth of various newly isolated strains of the invention and previously isolated strains, at 25°C and at 30°C. Growth rates are normalized to the growth rate of strain U-30 at 25°C. Previously isolated strains are designated by their ATCC accession numbers. Numerical data in terms of cell number doublings per day are given in Table 5.

Figure 6 is a graph of total yields of cellular production after induction by nitrogen limitation. Each of ash-free dry weight, total fatty acids and omega-3 highly unsaturated fatty acids, as indicated, was plotted, normalized to the corresponding value for strain 28211. All strains are identified by ATCC accession numbers.

Figure 7 is a graph of fatty acid yields after growth in culture media having the salinity indicated on the abscissa. Strains shown are newly isolated strains S31 (ATCC 20888) (□) and U42-2 (ATCC 20891) (+) and previously isolated strains, ATCC 28211 (◇) and ATCC 28209 (△). Fatty acid yields are plotted as relative yields normalized to an arbitrary value of 1.00 based on the average growth rate exhibited by S31 (ATCC 20888) (□) over the tested salinity range.

Figure 8 is a graph of increases in the omega-3 highly unsaturated fatty acid content of the total lipids in the brine shrimp, Artemia salina, fed

Thraustochytrid strain (ATCC 20890) isolated by the method in Example 1. EPA = C20:5w3; DHA = C22:6w3.

Figure 9 is a graph of increases in the omega-3 highly unsaturated fatty acid content of the total lipids in the brine shrimp, Artemia salina, fed Thraustochytrid strain (ATCC 20888) isolated by the method in Example 1. EPA = C20:5w3; DHA = C22:6w3.

EXAMPLES

Example 1. Collection and Screening

A 150ml water sample was collected from a shallow, inland saline pond and stored in a sterile polyethylene bottle. Special effort was made to include some of the living plant material and naturally occurring detritus (decaying plant and animal matter) along with the water sample. The sample was placed on ice until return to the laboratory. In the lab, the water sample was shaken for 15-30 seconds, and 1-10ml of the sample was pipetted or poured into a filter unit containing 2 types of filters: 1) on top, a sterile 47mm diameter Whatman #4 filter having a pore size about 25μm; and 2) underneath the Whatman filter, a 47mm diameter polycarbonate filter with about 1.0μm pore size. Given slight variations of nominal pore sizes for the filters, the cells collected on the polycarbonate filter range in size from about 1.0μm to about 25μm.

The Whatman filter was removed and discarded. The polycarbonate filter was placed on solid F-1 media in a petri plate, said media consisting of (per liter): 600ml seawater (artificial seawater can be used), 400ml distilled water, 10g agar, 1g glucose, 1g protein hydrolysate, 0.2g yeast extract, 2ml 0.1 M KH_2PO_4, 1ml of a vitamin solution (A-vits) (Containing 100mg/l thiamine, 0.5mg/l biotin, and 0.5mg/l cyanocobalamin), 5ml of a trace metal mixture (PII metals, containing per liter: 6.0g Na_2EDTA, 0.29g $FeCl_36H_2O$, 6.84g H_3BO_3, 0.86 $MnCl_24H_2O$, 0.06g $ZnCl_2$, 0.026g $CoCl_26H_2O$, (0.052g $NiSO_4H_2O$, 0.002g $CuSo_45H_2O$, and 0.005g $Na_2MoO_42H_2O$, and

500mg each of streptomycin sulfate and penicillin-G. The agar plate was incubated in the dark at 30°C. After 2-4 days numerous colonies appeared on the filter. Colonies of unicellular fungi (except yeast) were picked from the plate and restreaked on a new plate of similar media composition. Special attention was made to pick all colonies consisting of colorless or white cells. The new plate was incubated at 30°C and single colonies picked after a 2-4 day incubation period. Single colonies were then picked and placed in 50ml of liquid medium containing the same organic enrichments as in the agar plates. These cultures were incubated for 2-4 days at 30°C on a rotary shaker table (100-200 rpm). When the cultures appeared to reach maximal density, 20-40ml of the culture was harvested, centrifuged and lyophilized. The sample was then analyzed by standard, well-known gas chromatographic techniques (e.g., Lepage and Roy, 1984) to identify the fatty acid content of the strain. Those strains with omega-3 highly unsaturated fatty acids were thereby identified, and cultures of these strains were maintained for further screening.

Using the collection and screening process outlined above, over 150 strains of unicellular fungi have been isolated which have omega-3 highly unsaturated fatty acid contents up to 32% total cellular ash-free dry weight, and which exhibit growth over a temperature range from 15-48°C. Strains can also be isolated which have less than 1% (as % of total fatty acids) of the undesirable C20:4w6 and C22:5w6 highly unsaturated fatty acids. Strains of these fungi can be repeatedly isolated from the same location using the procedure outlined above. A few of the newly isolated strains have very similar fatty acid profiles. The possibility that some are duplicate isolates of the same strain cannot be ruled out at present. Further screening for other desirable traits such as salinity tolerance or ability to use a variety of carbon and nitrogen sources can then be carried out using a similar process.

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Example 2. Maintaining unrestricted cell growth: phosphorus

Cells of Thraustochytrium sp. U42-2 (ATCC No. 20891), a strain isolated by the method in Example 1, were picked from solid F-medium and inoculated into 50ml of modified FFM medium (Fuller et al., 1964). This medium containing: seawater, 1000ml; glucose, 1.0g; gelatin hydrolysate, 1.0g; liver extract, 0.01g; yeast extract, 0.1g; PII metals, 5ml; 1ml B-vitamins solution (Goldstein et al., 1969); and 1ml of an antibiotic solution (25g/l streptomycin sulfate and penicillin-G). 1.0ml of the vitamin mix (pH 7.2) contains: thiamine HCl, 200μg; biotin, 0.5μg; cyanocobalamin, 0.05μg; nicotinic acid, 100μg; calcium pantothenate, 100μg; riboflavin, 5.0μg; pyridoxine HCl, 40.0μg; pyridoxamine 2HCl, 20.0μg; p-aminobenzoic acid, 10μg; chlorine HCl, 500μg; inositol, 1.0mg; thymine, 0.8mg; orotic acid, 0.26mg; folinic acid, 0.2μg; and folic acid, 2.5μg. 250ml erlenmeyer flasks with 50ml of this medium were placed on an orbital shaker (200 rpm) at 27°C for 2-4 days, at which time the culture had reached their highest densities. One ml of this culture was transferred to a new flask of modified FFM medium, with the extra addition of one of the following treatments on a per liter basis: 1) 1ml of the B-vitamin mix; 2) 1ml of A-vitamin solution; 3) 5ml PII Metals; 4) 2ml of 0.1 M KH_2PO_2 (≈28mg); 5) treatments 2, 3, and 4 combined; and 6) 480mg KH_2PO_4. One ml of the culture was also transferred to a flask of modified FFM medium which had no extra additions made to it and served as a control for the experiment. The cultures were incubated for 48 hr. at 27°C on a rotary shaker (200 rpm). The cells were then harvested by centrifugation and the fatty acids were quantified by gas chromatography. The results are illustrated in Figure 1 and Table 1. In Figure 1, the yields are plotted as ratios of the control, whose relative yield is therefore 1.0.

Treatments 1-6 are as follows: 1) 2x concentration of B vitamins; 2) 2x concentration of A vitamins; 3) 2x concentration of trace metals; 4) 2x concentration of (B vitamins + phosphate + trace metals); 5) 2x concentration of phosphate; and 6) 24 mg phosphate per 50ml (.48g per liter). Only the treatment of adding 0.48g KH_2PO_4 per liter resulted in enhanced growth and resulted in significantly increased fatty acid yield.

Table 1. Effect of various nutrient additions on the yield of fatty acids in Thraustochytrium sp. U42-2 (ATCC No. 20891)

Treatment	Fatty Acid Yield mg/liter
Control	23
2x concentration of B vitamin mix	17
2x concentration of A vitamin mix	24
2x concentration trace metals	27
2x concentration B vitamin mix, 2x PO_4 and 2x concentration trace metals	24
2x concentration PO_4	23
24mg phosphate per 50 ml	45

Example 3. Maintaining unrestricted growth: PO_4 and yeast extract

Cells of Schizochytrium aggregatum (ATCC 28209) were picked from solid F-1 medium and inoculated into 50ml of FFM medium. The culture was placed on a rotary shaker (200 rpm) at 27°C. After 3-4 days, 1ml of this culture was transferred to 50ml of each of the following treatments: 1) FFM medium (as control); and 2) FFM medium with the addition of 250mg/l KH_2PO_4 and 250mg/l yeast extract. These cultures were placed on a rotary shaker (200 rpm) at 27°C for 48 hr. The cells were harvested and the yield of cells quantified. In treatment 1, the final concentration of cells on an ash-free dry weight basis was 616mg/l. In treatment 2, the final concentration of cells was 1675mg/l, demonstrating the enhanced effect of increasing PO_4 and yeast extract concentrations in the culture medium.

Example 4. Maintaining unrestricted growth: substitution of corn steep liquor for yeast extract

Cells of Schizochytrium sp. S31 (ATCC No. 20888) were picked from solid F-1 medium and placed into 50ml of M-5 medium. This medium consists of (on a per liter basis): NaCl, 25g; $MgSO_4 \cdot 7H_2O$, 5g; KCl, 1g; $CaCl_2$, 200mg; glucose, 5g; glutamate, 5g; KH_2PO_4, 1g; PII metals, 5ml; A-vitamins solution, 1ml; and antibiotic solution, 1ml. The pH of the solution was adjusted to 7.0 and the solution was filter sterilized. Sterile solutions of corn steep liquor (4g/40ml; pH 7.0) and yeast extract 1g/40ml; pH 7.0) were prepared. To one set of M-5 medium flasks, the following amount of yeast extract solution was added: 1) 2ml; 2) 1.5ml; 3) 1ml; 4) 0.5ml; and 5) 0.25ml. To another set of M-5 medium flasks the yeast extract and corn steep liquor solutions were added at the following levels: 1) 2ml yeast extract; 2) 1.5ml yeast extract and 0.5ml corn steep liquor; 3) 1.0ml yeast extract and 1.0ml corn steep liquor; 4) 0.5ml yeast extract and 1.5ml corn steep

liquor; and 5) 2ml corn steep liquor. One ml of the culture in F-1 medium was used to inoculate each flask. They were placed on a rotary shaker at 27°C for 48 hr. The cells were harvested by centrifugation and the yield of cells (as ash-free dry weight) was determined. The results are shown in Table 2. The results indicate the addition of yeast extract up to 0.8g/l of medium can increase the yield of cells. However, addition of corn steep liquor is even more effective and results in twice the yield of treatments with added yeast extract. This is very advantageous for the economic production of cells as corn steep liquor is much less expensive than yeast extract.

Table 2

Treatment (Amount Nutrient Supplement Added)	Ash-Free Dry Weight (mg/l)
2.0ml yeast ext.	4000
1.5ml yeast ext.	4420
1.0ml yeast ext.	4300
0.5ml yeast ext.	2780
0.25ml yeast ext.	2700
2.0ml yeast ext.	4420
1.5ml yeast ext. + 0.5ml CSL*	6560
1.0ml yeast ext. + 1.0ml CSL	6640
0.5ml yeast ext. + 1.5ml CSL	7200
2.0ml CSL	7590

*CSL = corn steep liquor

Example 5. Enhanced highly unsaturated fatty acid content of strains isolated by method in Example 1 compared to ATCC strains (previously known strains)

A battery of 151 newly isolated strains, selected according to the method described in Example 1, were sampled in late exponential phase growth and quantitatively analyzed for highly unsaturated fatty acid content by gas-liquid chromatography. All strains were grown either in M1 medium or liquid FFM medium, whichever gave highest yield of cells. Additionally, five previously isolated Thraustochytrium or Schizochytrium species were obtained from the American Type Culture Collection, representing all the strains which could be obtained in viable form from the collection. These strains were: T. aureum (ATCC No. 28211), T. aureum (ATCC No. 34304), T. roseum (ATCC No. 28210), T. straitum (ATCC No. 34473) and S. aggregatum (ATCC No. 28209). The strains all exhibited abbreviated growth in conventional media, and generally showed improved growth in media of the present invention, including M5 medium and FFM medium, Example 2. The fatty acids production of each of the known strains was measured as described, based upon the improved growth of the strains in media of the invention.

Fatty acid peaks were identified by the use of pure compounds of known structure. Quantitation, in terms of percent by weight of total fatty acids, was carried out by integrating the chromatographic peaks. Compounds identified were: palmitic acid (C16:0), C20:4w6 and C22:1 (which were not resolved separately by the system employed), C20:5w3, C22:5w6, C22:5w3, and C22:6w3. The remainder, usually lower molecular weight fatty acids, were included in the combined category of "other fatty acids." Total omega-3 fatty acids were calculated as the sum of 20:5w3, 22:5w3 and 22:6w3. Total omega-6 fatty acids were calculated as the sum of the 20:4/22:1 peak and the 22:5w6 peak.

The results are shown in Tables 3-4 and illustrated in Figs. 2-4. From Table 3 it can be seen that large numbers of strains can be isolated by the method of the invention, and that large numbers of strains outperform the previously known strains by several important criteria. For example, 102 strains produced at least 7.8% by weight of total fatty acids C20:5w3, a higher percentage of that fatty acid than any previously known strain. Strains 23B (ATCC No. 20892) and 12B (ATCC No. 20890) are examples of such strains. Thirty (30) strains of the invention produced at least 68% by weight of total fatty acids as omega-3 fatty acids, more than any previously known strain. Strain 23B (ATCC No. 20892) is an example of such strains. Seventy-six (76) strains of the invention yielded not more than 10% by weight of total fatty acids as omega-6 fatty acids, considered undesirable components of the human diet, lower than any previously known strain. Strains 23B (ATCC No. 20892) and 12B (ATCC No. 20890) are examples of such strains. In addition, there are 35 strains of the invention that produce more than 25% by weight of total fatty acids as omega-6 fatty acids, more than any previously known strain. While such strains may not be useful for dietary purposes, they are useful as feedstock for chemical synthesis of eicosanoids starting from omega-6 fatty acids.

In addition, the data reveal many strains of the invention which produce a high proportion of total omega-3 fatty acids as C22:6w3. In Table 4, 48 of the strains shown in Table 2 were compared to the previously known strains, showing each of C20:5w3, C22:5w3 and C22:6w3 as percent by weight of total omega-3 content. Fifteen strains had at least 94% by weight of total omega-3 fatty acids as C22:6w3, more than any previously known strain. Strain S8 (ATCC No. 20889) was an example of such strains. Eighteen strains had at least 28% by weight of total omega-3 fatty acids as C20:5w3, more

than any previously known strain. Strain 12B (ATCC No. 20890) was an example of such strains.

Figure 2 illustrates the set of strains, isolated by the method in Example 1, that have more than 67% omega-3 fatty acids (as % of total fatty acids) and less than 10.6% omega-6 fatty acids (as % of total fatty acids). All of the previously known strains had less than 67% omega-3 fatty acids (as % of total fatty acids) and greater than 10.6% omega-6 (as % of total fatty acids).

Figure 3 illustrates the set of strains, isolated by the method in Example 1, that have more than 67% omega-3 fatty acids (as % of total fatty acids) and greater than 7.5% C20:5w3 (as % of total fatty acids). All of the previously known strains had less than 67% omega-3 fatty acids (as % of total fatty acids) and less than 7.8% C20:5w3 (as % of total fatty acids).

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TABLE 3: LIST OF STRAINS AND COMPOSITIONS UNDER STANDARD SCREENING CONDITIONS

PER CENT OF TOTAL FATTY ACIDS							Total	Total	Strain
C16:0	C20:4w6	C20:5w3	C22:5w6	C22:5w3	C22:6w3	Other FA	Omega3	Omega6	
30.4%	2.8%	6.6%	3.2%	0.2%	8.3%	48.5%	15.1%	6.0%	21
22.9%	0.4%	2.3%	15.5%	0.5%	47.0%	11.5%	49.7%	15.9%	ATCC20889
14.9%	6.5%	12.0%	11.8%	0.4%	49.7%	4.7%	62.1%	18.3%	U40-2
40.3%	1.7%	3.8%	8.6%	0.0%	8.2%	37.4%	12.0%	10.2%	21B
20.7%	0.4%	7.8%	0.0%	0.0%	1.1%	70.1%	8.9%	0.4%	BG1
26.0%	5.7%	1.5%	9.7%	0.7%	9.7%	46.7%	11.9%	15.4%	56A
16.4%	1.4%	10.0%	1.9%	2.2%	46.4%	21.8%	58.6%	3.3%	11A-1
23.7%	3.3%	10.5%	1.9%	1.8%	29.9%	28.9%	42.2%	5.2%	4A-1
18.7%	6.9%	9.2%	11.9%	3.2%	25.2%	24.9%	37.5%	18.8%	17B
15.4%	4.2%	7.3%	9.5%	0.9%	51.2%	11.6%	59.3%	13.7%	ATCC20891
22.3%	3.9%	7.6%	23.5%	0.5%	22.1%	20.2%	30.2%	27.4%	S44
14.4%	2.3%	15.0%	18.4%	0.7%	43.8%	5.5%	59.4%	20.7%	U30
22.1%	7.8%	3.1%	12.7%	1.0%	14.9%	38.3%	19.0%	20.5%	59A
18.1%	2.3%	6.9%	9.1%	0.8%	52.2%	10.6%	59.9%	11.4%	U37-2
15.8%	3.9%	8.8%	11.6%	1.2%	53.3%	5.5%	63.3%	15.5%	S50W
23.7%	3.8%	6.3%	6.9%	0.6%	43.0%	15.6%	50.0%	10.7%	ATCC20891
10.0%	0.0%	0.0%	0.0%	0.0%	0.0%	90.0%	0.0%	0.0%	UX
16.6%	6.3%	11.9%	13.3%	1.7%	43.0%	7.3%	56.6%	19.5%	LW9
17.3%	2.3%	8.4%	11.4%	0.7%	53.6%	6.5%	62.6%	13.6%	C32-2
23.8%	1.2%	6.4%	2.5%	1.9%	34.4%	29.8%	42.6%	3.7%	5A-1
17.1%	5.2%	11.1%	7.6%	2.2%	27.2%	29.6%	40.4%	12.9%	BG1
25.4%	2.2%	9.6%	7.0%	1.1%	46.0%	8.8%	56.7%	9.1%	U3
16.9%	12.0%	6.6%	16.2%	0.4%	25.1%	22.8%	32.1%	28.2%	55B
26.3%	2.6%	8.6%	2.0%	2.5%	32.4%	25.5%	43.5%	4.6%	18A
19.4%	0.3%	9.8%	0.0%	0.3%	38.4%	31.7%	48.6%	0.3%	32B
16.0%	16.7%	8.6%	18.4%	0.0%	22.5%	17.7%	31.1%	35.1%	56B
18.6%	7.7%	11.4%	3.6%	4.3%	31.7%	22.7%	47.4%	11.2%	SX2
17.8%	4.4%	16.2%	6.4%	3.7%	33.6%	17.8%	53.5%	10.9%	53B
16.8%	2.7%	13.8%	20.5%	1.4%	39.3%	5.5%	54.4%	23.3%	S49
20.8%	8.0%	8.9%	6.4%	1.7%	33.9%	20.3%	44.5%	14.4%	S3
14.8%	0.3%	3.7%	3.9%	0.0%	69.9%	7.4%	73.6%	4.2%	3A-1
28.1%	5.2%	12.7%	3.2%	0.9%	20.9%	29.0%	34.5%	8.4%	15A
20.9%	0.7%	8.5%	1.0%	0.0%	35.8%	33.0%	44.3%	1.7%	9A-1
15.7%	10.2%	8.8%	13.4%	1.5%	23.9%	26.3%	34.3%	23.7%	51B
16.2%	11.2%	7.8%	16.4%	1.5%	20.4%	26.5%	29.7%	27.6%	8A-1
20.5%	5.5%	8.6%	4.8%	2.7%	28.7%	29.2%	40.0%	10.3%	13A-1
16.1%	13.6%	11.1%	16.0%	0.0%	28.4%	14.8%	39.4%	29.6%	24B-2
16.9%	7.3%	16.4%	6.1%	0.0%	40.8%	12.4%	57.2%	13.4%	24B-1
16.2%	0.0%	10.9%	1.0%	0.0%	56.5%	15.5%	67.4%	1.0%	3B
17.0%	0.0%	5.0%	2.3%	0.0%	73.4%	2.3%	78.3%	2.3%	SBG5
20.8%	4.5%	5.8%	3.8%	1.0%	22.7%	41.3%	29.5%	8.4%	16B
19.0%	14.0%	8.3%	18.9%	0.7%	23.9%	15.2%	32.9%	32.9%	6A-1
18.0%	0.3%	10.1%	0.0%	0.0%	48.9%	22.7%	59.0%	0.3%	33B

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PER CENT OF TOTAL FATTY ACIDS							Total	Total	Strain
C16:0	C20:4w6	C20:5w3	C22:5w6	C22:5w3	C22:6w3	Other FA	Omega3	Omega6	
16.7%	5.5%	14.8%	8.5%	1.7%	31.8%	21.0%	48.3%	13.9%	B40
15.0%	1.0%	11.7%	2.1%	0.9%	62.3%	6.9%	74.9%	3.1%	28A
17.8%	18.5%	8.1%	20.5%	0.0%	22.1%	12.9%	30.2%	39.0%	43B
16.9%	0.0%	3.4%	2.7%	0.0%	61.2%	15.8%	64.6%	2.7%	1A-1
15.6%	2.7%	11.4%	10.9%	0.8%	53.7%	4.9%	65.9%	13.6%	U41-2
16.5%	0.7%	3.9%	3.9%	0.0%	68.4%	6.7%	72.2%	4.6%	56B
14.4%	0.9%	10.9%	2.5%	1.0%	66.4%	3.8%	78.3%	3.4%	46A
17.6%	0.0%	2.4%	3.3%	0.0%	66.3%	10.4%	68.7%	3.3%	15A-1
25.0%	0.0%	3.3%	0.0%	1.4%	53.2%	17.1%	57.9%	0.0%	13A
16.1%	13.4%	9.3%	13.6%	0.0%	32.3%	15.3%	41.6%	27.0%	37B
16.5%	9.1%	13.2%	6.7%	0.0%	38.9%	15.6%	52.1%	15.9%	43B
16.1%	12.4%	12.0%	15.7%	0.8%	30.5%	12.5%	43.3%	28.1%	17B
13.8%	0.8%	11.5%	2.8%	0.0%	67.0%	4.1%	78.6%	3.6%	27A
17.5%	18.6%	9.0%	19.5%	0.0%	21.7%	13.7%	30.7%	38.1%	46B
21.4%	1.4%	18.9%	0.0%	5.0%	43.5%	9.9%	67.3%	1.4%	ATCC20890
17.7%	0.0%	0.6%	4.4%	0.0%	68.2%	9.1%	68.8%	4.4%	5A
17.6%	16.0%	9.6%	18.8%	0.0%	25.6%	12.4%	35.2%	34.8%	28B-2
14.0%	0.9%	13.2%	1.6%	0.0%	64.7%	5.5%	77.9%	2.6%	27B
19.5%	2.9%	16.6%	1.1%	1.6%	30.2%	28.1%	48.5%	4.0%	49B
17.2%	0.7%	6.8%	2.7%	0.0%	63.0%	9.6%	69.8%	3.4%	18B
14.4%	3.5%	13.5%	26.0%	1.0%	37.2%	4.4%	51.6%	29.5%	S49-2
16.1%	2.2%	15.7%	21.6%	0.0%	36.7%	7.8%	52.4%	23.7%	20B
17.3%	4.7%	14.3%	7.2%	2.9%	30.2%	23.5%	47.3%	11.9%	8B
11.5%	3.3%	11.3%	6.5%	1.1%	59.9%	6.5%	72.2%	9.8%	13B
16.6%	0.7%	10.7%	1.6%	0.0%	59.7%	10.8%	70.4%	2.2%	26A
16.1%	3.3%	13.5%	23.8%	0.0%	38.7%	4.7%	52.2%	27.1%	S42
15.6%	0.6%	12.1%	0.0%	0.0%	60.2%	11.5%	72.3%	0.6%	35B
19.5%	0.0%	1.4%	3.4%	0.0%	66.6%	9.1%	68.0%	3.4%	42A
18.9%	3.5%	12.7%	25.0%	0.0%	35.0%	5.0%	47.6%	28.5%	40A
25.2%	3.3%	9.3%	21.8%	0.0%	30.3%	10.1%	39.6%	25.1%	S50C
17.6%	11.1%	13.2%	14.1%	1.3%	28.7%	14.0%	43.2%	25.2%	59A
19.9%	0.0%	5.5%	1.9%	0.0%	66.8%	6.0%	72.3%	1.9%	S8G9
15.4%	3.1%	13.2%	26.1%	0.0%	35.8%	6.5%	49.1%	29.1%	21B
18.9%	0.7%	11.6%	0.0%	0.0%	59.1%	9.7%	70.7%	0.7%	2B
14.1%	1.1%	12.4%	2.0%	0.0%	65.2%	5.2%	77.6%	3.1%	1B
22.2%	16.2%	6.3%	17.7%	0.0%	18.1%	19.5%	24.4%	33.8%	55B
16.0%	1.0%	4.5%	0.0%	0.0%	69.5%	9.0%	74.0%	1.0%	3A
17.0%	4.3%	12.4%	29.8%	0.0%	34.0%	2.5%	46.4%	34.1%	9B
15.4%	4.3%	8.7%	13.2%	0.0%	53.2%	5.1%	62.0%	17.5%	U24
14.2%	3.1%	12.0%	20.0%	1.1%	35.2%	14.3%	48.3%	23.2%	U28
16.8%	14.6%	10.1%	16.0%	0.6%	27.7%	14.0%	38.5%	30.7%	28B-1
23.2%	1.9%	8.3%	1.1%	2.3%	22.7%	40.4%	33.3%	3.0%	44B
24.6%	15.8%	8.7%	16.0%	0.0%	15.3%	19.6%	24.0%	31.8%	54B
15.5%	0.0%	1.3%	2.9%	0.0%	72.7%	7.6%	74.0%	2.9%	55A
18.4%	1.0%	5.0%	3.0%	0.0%	66.2%	6.4%	71.3%	3.9%	49A
18.6%	15.3%	9.4%	18.0%	0.0%	27.3%	11.4%	36.7%	33.3%	51A
23.5%	13.1%	7.3%	17.9%	0.0%	26.7%	11.4%	34.0%	31.0%	14A-1
13.3%	1.1%	14.5%	0.9%	0.0%	64.6%	5.6%	79.1%	2.0%	25B
22.9%	2.4%	10.3%	21.5%	0.0%	26.5%	16.4%	36.9%	23.9%	41A
16.8%	1.0%	9.7%	2.7%	0.0%	58.3%	11.5%	68.0%	3.7%	24A
0.4%	8.5%	14.1%	10.2%	2.1%	27.6%	37.0%	43.8%	18.8%	61A

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PER CENT OF TOTAL FATTY ACIDS							Total	Total	Strain
C16:0	C20:4w6	C20:5w3	C22:5w6	C22:5w3	C22:6w3	Other FA	Omega3	Omega6	
30.5%	0.0%	7.1%	0.0%	0.0%	0.6%	61.8%	7.7%	0.0%	BRBG
18.2%	14.9%	8.3%	18.7%	0.0%	24.4%	15.5%	32.7%	33.6%	17A
17.4%	2.0%	9.3%	2.8%	0.0%	55.7%	12.7%	65.0%	4.9%	60A
14.1%	0.8%	13.0%	1.2%	0.0%	67.8%	3.1%	80.8%	2.0%	26B
17.8%	5.0%	6.9%	15.0%	1.5%	47.4%	6.4%	55.8%	20.0%	ATCC20888
16.0%	0.0%	1.8%	2.0%	0.0%	70.8%	9.4%	72.6%	2.0%	2A
24.6%	0.0%	4.0%	0.0%	0.0%	49.4%	22.0%	53.4%	0.0%	44A
17.4%	1.8%	0.0%	2.9%	0.0%	55.3%	23.3%	55.3%	4.6%	14A
23.3%	1.3%	4.6%	0.0%	0.0%	12.6%	58.1%	17.3%	1.3%	41B
19.3%	0.0%	1.1%	3.8%	0.0%	66.6%	9.1%	67.8%	3.8%	66A
18.6%	15.6%	8.3%	17.1%	1.1%	24.6%	14.8%	33.9%	32.7%	11A
19.6%	5.1%	10.1%	27.2%	0.0%	27.5%	10.6%	37.5%	32.3%	2X
15.7%	2.4%	14.0%	25.7%	0.0%	36.7%	5.4%	50.8%	28.1%	33A
14.6%	1.5%	13.5%	0.0%	0.0%	66.0%	4.3%	79.5%	1.5%	ATCC20892

PRIOR STRAINS

PER CENT OF TOTAL FATTY ACIDS							Total	Total	Strain
C16:0	C20:4w6	C20:5w3	C22:5w6	C22:5w3	C22:6w3	Other FA	Omega3	Omega6	
15.7%	3.9%	3.7%	8.1%	0.0%	55.1%	13.5%	58.8%	12.0%	ATCC34304
28.2%	1.6%	6.9%	11.4%	0.0%	17.8%	34.1%	24.7%	12.9%	ATCC24473
15.2%	2.9%	7.7%	9.8%	0.6%	54.6%	9.2%	62.9%	12.7%	ATCC28211
23.2%	10.7%	4.3%	12.6%	1.5%	20.6%	27.0%	26.4%	23.4%	ATCC28209
13.2%	6.3%	6.9%	4.3%	0.0%	60.1%	9.1%	67.0%	10.6%	ATCC28210

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T470X

TABLE 4: COMPOSITION OF OMEGA 3 FATTY ACID FRACTION

EPA C20:5w3	DPA C22:5w3	DHA C22:6w3	Strain
44.0%	1.1%	54.9%	21
4.6%	0.9%	94.5%	ATCC20889
19.3%	0.7%	80.0%	U40-2
31.9%	0.0%	68.1%	21B
87.9%	0.0%	12.1%	BRBG1
12.5%	6.1%	81.5%	56A
17.0%	3.7%	79.3%	11A-1
24.9%	4.3%	70.8%	4A-1
24.4%	8.4%	67.2%	17B
12.2%	1.5%	86.3%	ATCC20891
25.1%	1.7%	73.2%	S44
25.2%	1.1%	73.7%	U30
16.2%	5.4%	78.4%	59A
11.5%	1.4%	87.1%	U37-2
14.0%	1.9%	84.2%	S50W
12.7%	1.3%	86.0%	ATCC20891
---	---	---	UX
21.0%	2.9%	76.1%	LWN9
13.4%	1.0%	85.6%	C32-2
15.0%	4.3%	80.7%	5A-1
27.4%	5.4%	67.2%	BRBG1
17.0%	1.9%	81.1%	U3
20.5%	1.3%	78.2%	55B
19.8%	5.8%	74.4%	18A
20.1%	0.7%	79.2%	32B
27.8%	0.0%	72.2%	56B
24.1%	9.1%	66.9%	SX2
30.3%	6.9%	62.8%	53B
25.3%	2.5%	72.2%	S49
19.9%	3.8%	76.3%	S3
5.0%	0.0%	95.0%	3A-1
36.9%	2.6%	60.5%	15A
19.3%	0.0%	80.7%	9A-1
25.8%	4.4%	69.8%	51B
26.3%	5.0%	68.7%	8A-1
21.6%	6.7%	71.7%	13A-1
28.0%	0.0%	72.0%	24B-2
28.7%	0.0%	71.3%	24B-1
16.2%	0.0%	83.8%	3B
6.3%	0.0%	93.7%	SBG5
19.7%	3.3%	77.0%	16B
25.2%	2.1%	72.6%	6A-1
17.1%	0.0%	82.9%	33B
30.5%	3.6%	65.9%	B40
15.6%	1.2%	83.1%	28A

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EPA C20:5w3	DPA C22:5w3	DHA C22:6w3	Strain
26.8%	0.0%	73.2%	43B
5.2%	0.0%	94.8%	1A-1
17.4%	1.2%	81.5%	U41-2
5.4%	0.0%	94.6%	56B
13.9%	1.3%	84.8%	46A
3.5%	0.0%	96.5%	15A-1
5.8%	2.4%	91.8%	13A
22.3%	0.0%	77.7%	37B
25.4%	0.0%	74.6%	43B
27.7%	1.9%	70.3%	17B
14.7%	0.0%	85.3%	27A
29.2%	0.0%	70.8%	46B
28.0%	7.5%	64.5%	ATCC20890
0.9%	0.0%	99.1%	5A
27.3%	0.0%	72.7%	28B-2
16.9%	0.0%	83.1%	27B
34.3%	3.4%	62.3%	49B
9.7%	0.0%	90.3%	18B
26.1%	1.9%	71.9%	S49-2
29.9%	0.0%	70.1%	20B
30.1%	6.2%	63.7%	8B
15.6%	1.5%	82.9%	13B
15.2%	0.0%	84.8%	26A
25.9%	0.0%	74.1%	S42
16.7%	0.0%	83.3%	35B
2.1%	0.0%	97.9%	42A
26.6%	0.0%	73.4%	40A
23.4%	0.0%	76.6%	S50C
30.6%	2.9%	66.4%	S9A
7.6%	0.0%	92.4%	S8G9
27.0%	0.0%	73.0%	21B
16.4%	0.0%	83.6%	2B
15.9%	0.0%	84.1%	1B
25.9%	0.0%	74.1%	S5B
6.0%	0.0%	94.0%	3A
26.7%	0.0%	73.3%	9B
14.1%	0.0%	85.9%	U24
24.9%	2.2%	72.9%	U28
26.4%	1.5%	72.1%	28B-1
24.8%	6.9%	68.3%	44B
36.4%	0.0%	63.6%	54B
1.8%	0.0%	98.2%	55A
7.1%	0.0%	92.9%	49A
25.6%	0.0%	74.4%	51A
21.5%	0.0%	78.5%	14A-1
18.4%	0.0%	81.6%	25B
28.1%	0.0%	71.9%	41A
14.3%	0.0%	85.7%	24A
32.3%	4.8%	63.0%	61A
91.6%	0.0%	8.4%	BRBG

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EPA C20:5w3	DPA C22:5w3	DHA C22:6w3	Strain
25.5%	0.0%	74.5%	17A
14.4%	0.0%	85.6%	60A
16.1%	0.0%	83.9%	26B
12.4%	2.7%	84.9%	ATCC20888
2.5%	0.0%	97.5%	2A
7.5%	0.0%	92.5%	44A
0.0%	0.0%	100.0%	14A
26.7%	0.0%	73.3%	41B
1.7%	0.0%	98.3%	66A
24.5%	3.1%	72.4%	11A
26.8%	0.0%	73.2%	2X
27.6%	0.0%	72.4%	33A
17.0%	0.0%	83.0%	ATCC20892

PRIOR STRAINS

EPA C20:5w3	DPA C22:5w3	DHA C22:6w3	Strain
6.4%	0.0%	93.6%	ATCC34304
27.9%	0.0%	72.1%	ATCC24473
12.2%	1.0%	86.8%	ATCC28211
16.4%	5.6%	78.1%	ATCC28209
10.3%	0.0%	89.7%	ATCC28210

Example 6. Enhanced growth rates of strains isolated by method in Example 1 compared to ATCC strains (previously known strains

Cells of Schizochytrium sp. S31 (ATCC No. 20888), Schizochytrium sp. S8 (ATCC No. 20889), Thraustochytrium sp. S42, Thraustochytrium sp. U42-2, Thraustochytrium sp. S42 and U30, (all isolated by the method of Example 1) and Thraustochytrium aureum (ATCC #28211) and Schizochytrium aggregatum (ATCC #28209) (previously known strains) were picked from solid F-1 medium and placed into 50ml of M-5 medium. This medium consists of (on a per liter basis): Yeast Extract, 1g; NaCl, 25g; $MgSO_4 \cdot 7H_2O$, 5g; KCl, 1g; $CaCl_2$, 200mg; glucose, 5g; glutamate, 5g; KH_2PO_4, 1g; PII metals, 5ml; A-vitamins solution, 1ml; and antibiotic solution, 1ml. The pH of the solution was adjusted to 7.0 and the solution was filter sterilized. After three days of growth on an orbital shaker (200 rpm, 27°C), 1-2ml of each culture was transferred to another flask of M-5 medium and placed on the shaker for 2 days. The cultures (1-2ml) were then transferred to another flask of M-5 medium and placed on the shaker for 1 day. This process ensured that all cultures were in the exponential phase of growth. These later cultures were then used to inoculate two 250ml flasks of M-5 medium for each strain. These flasks were than placed on shakers at 25°C and 30°C, and changes in their optical density were monitored on a Beckman DB-G spectrophotometer (660nm, 1cm path length). Optical density readings were taken at the following times: 0, 6, 10, 14, 17.25, 20.25 and 22.75 hours. Exponential growth rates (doublings/day) were then calculated from the optical density data by the method of Sorokin (1973). The results are presented in Table 5 and illustrated (normalized to the growth of strain U30 at 25°C) in Fig. 5. The data indicate that the strains isolated by the method in Example 1 have much higher growth rates than the previously known ATCC

strains at both 25°C and 30°C, even under the optimized phosphate levels essential for continuous growth. Strains of Thraustochytriales isolated from cold Antarctic waters have not been shown to grow at 30°C.

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Table 5

Exponential Growth Rate
(doublings/day)

Strain	25°C	30°C
S31*	8.5	9.4
U40-2*	5.8	6.0
S8*	7.1	8.8
S42*	6.6	8.3
U30*	5.5	7.3
28209**	4.6	5.0
28210**	3.5	4.5
28211**	4.2	5.7
34304**	2.7	3.7
24473**	4.6	5.3

* strain isolated by method in Example 1

** previously known ATCC strain

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Example 7. Enhanced production characteristics (growth and lipid induction) of strains isolated by method in Example 1 compared to ATCC strains (prior art strains)

Cells of Schizochytrium sp. S31 (ATCC No. 20888), Schizochytrium sp. S8 (ATCC No. 20889) (both isolated by the method of Example 1) and Thraustochytrium aureum (ATCC #28211) and Schizochytrium aggregatum (ATCC #28209) (prior art strains) were picked from solid F-1 medium and placed into 50ml of M-5 medium (see Example 5). The pH of the solution was adjusted to 7.0 and the solution was filter sterilized. After three days of growth on an orbital shaker (200 rpm, 27°C), 1-2ml of each culture was transferred to another flask of M-5 medium and placed on the shaker for 2 days. The ash-free dry weights for each of these cultures were then quickly determined ~~that~~ and 3.29mg of each culture was pipetted into two 250ml erlenmeyer flasks containing 50ml of M-5 medium. These flasks were placed on a rotary shaker (200 rpm, 27°C). After 24 hours 20ml portions of each culture were then centrifuged, the supernatants discarded, and the cells transferred to 250ml erlenmeyer flasks containing 50 ml of M-5 medium without any glutamate (N-source). The flasks were placed back on the shaker, and after another 12 hours they were sampled to determine ash-free dry weights and quantify fatty acid contents by the method of Lepage and Roy (1984). The results are illustrated (normalized to the yields of ATCC No. 28211, previously known strain) in Fig. 6. The results indicate that the strains isolated by the method of Example 1 produced 2-3 times as much ash-free dry weight in the same period of time, under a combination of exponential growth and nitrogen limitation (for lipid induction) as the prior art ATCC strains. In addition, higher yields of total fatty acids and omega-3 fatty acids were obtained from strains of the present invention with strains S31 (ATCC No. 20888) producing 3-4 times as much omega-3 fatty acids as the prior art ATCC strains.

Example 8. Enhanced salinity tolerance and fatty acid production by strains isolated by method in Example 1

Strains of 4 species of ~~Oomycetes~~ Thraustochytrids, Schizochytrium sp. S31 (ATCC No. 20888) and Thraustochytrium sp. U42-2 (ATCC No. 20891) (both isolated and screened by the method of Example 1), and S. aggregatum (ATCC 28209) and T. aureum (ATCC 28210) (obtained from the American Type Culture Collection) were picked from solid F-1 medium and incubated for 3-4 days at 27°C on a rotary shaker (200 rpm). A range of differing salinity medium was prepared by making the following dilutions of M medium salts (NaCl, 25g/l; $MgSO_4 \cdot 7H_2O$, 5g/l; KCl, 1g/l; $CaCl_2$, 200mg/l: 1) 100% (w/v M medium salts; 2) 80% (v/v) M medium, 20% (v/v) distilled water; 3) 60% (v/v) M medium, 40% (v/v) distilled water; 4) 40% (v/v) M medium, 60% (v/v) distilled water; 5) 20% (v/v) M medium, 80% distilled water; 6) 15% (v/v) M medium, 85% (v/v) distilled water; 7) 10% (v/v) M medium, 90% (v/v) distilled water; 8) 7% (v/v) M medium, 93% (v/v) distilled water; 9) 3% (v/v) M medium, 97% (v/v) distilled water; 10) 1.5% (v/v) M medium, 98.5% (v/v) distilled water. The following nutrients were added to the treatments (per liter): glucose, 5g; glutamate, 5g; yeast ext., 1g; $(NH_4)_2SO_4$, 200 mg; $NaHCO_3$, 200 mg; KH_2PO_4, 1 g/l; PII metals, 5ml; A-vitamins solution, 1ml; and antibiotics solution, 2ml. Fifty ml of each of these treatments were inoculated with 1ml of the cells growing in the F-1 medium. These cultures were placed on an orbital shaker (200 rpm) and maintained at 27°C for 48 hr. The cells were harvested by centrifugation and total fatty acids determined by gas chromatography. The results are illustrated in Fig. 7. Thraustochytrium sp. U42-2 (ATCC No. 20891) isolated by the method of Example 1 can yield almost twice the amount of fatty acids produced by T. aureum (ATCC 28210) and over 8 times the amount of fatty acids produced by S. aggregatum (ATCC 28209). Additionally, U42-2 appears to have a wider salinity

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tolerance at the upper end of the salinity range evaluated. Schizochytrium sp. S31 (ATCC No. 20888), also isolated by the method in Example 1, exhibited both a high fatty acid yield (2.5 to 10 times that of the previously known ATCC strains) and a much wider range of salinity tolerance than the ATCC strains. Additionally, Schizochytrium sp. S31 (ATCC No. 20888) grows best at very low salinities. This property provides a strong economic advantage when considering commercial production, both because of the corrosive effects of saline waters on metal reactors, and because of problems associated with the disposal of saline waters.

Example 9. Cultivation/Low Salinity

Fifty ml of M/10-5 culture media in a 250ml erlenmeyer flask was inoculated with a colony of Schizochytrium sp. S31 (ATCC No. 20888) picked from an agar slant. The M/10-5 media contains: 1000ml deionized water, 2.5g NaCl, 0.5g $MgSO_4$ $7H_2O$, 0.1g KCl, 0.02g $CaCl_2$, 1.0g KH_2PO_4, 1.0g yeast extract, 5.0g glucose, 5.0g glutamic acids, 0.2g $NaHCO_3$, 5ml PII trace metals, 2ml vitamin mix, and 2ml antibiotic mix. The culture was incubated at 30°C on a rotary shaker (200 rpm). After 2 days the culture was at a moderate density and actively growing. 20ml of this actively growing culture was used to inoculate a 2 liter fermenter containing 1700ml of the same culture media except the concentration of the glucose and glutamate had been increased to 40g/l (M/10-40 media). The fermenter was maintained at 30°C, with aeration at 1 vol/vol/min, and mixing at 300 rpm. After 48 hr, the concentration of cells in the fermenter was 21.7g/l. The cells were harvested by centrifugation, lyophilized, and stored under N_2.

The total fatty acid content and omega-3 fatty acid content was determined by gas chromatography. The total fatty acid content of the final product was 39.0% ash-free dry weight. The omega-3 highly unsaturated fatty

acid content (C20:5w3, C22:5w3 and C22:6w3) of the microbial product was 25.6% of the ash-free dry weight. The ash content of the sample was 7.0%.

Example 10.

Growth and gas chromatographic analysis of fatty acid production by various strains as described in example 5 revealed differences in fatty acid diversity. Strains of the present invention synthesized fewer different fatty acids than previously available strains. Lower diversity of fatty acids is advantageous in fatty acid purification since there are fewer impurities to be separated. For food supplement purposes, fewer different fatty acids is advantageous because the likelihood of ingesting unwanted fatty acids is reduced. Table 6 shows the number of different highly unsaturated fatty acids present, at concentrations greater than 1% by weight of total fatty acids for previously known strains, designated by ATCC number and various strains of the present invention.

Table 6

Strain	No. of Different Fatty Acids at 1% or Greater % of Total Fatty Acids
34304**	8
28211**	8
24473**	10
28209**	13
28210**	8
S31*	5
S8*	6
79B*	6

* strain isolated by the method in Example 1

** previously known ATCC strain

Example 11. Recovery

Fifty ml of M5 culture media in a 250 ml erlenmeyer flask was inoculated with a colony of Schizochytrium sp. S31 (ATCC No. 20888) picked from an agar slant. The M5 media contains: 1000ml deionized water, 25.0g NaCl, 5.0g $MgSO4 \cdot 7H_2O$, 1.0g KCl, 0.2g $CaCl_2$, 1.0g KH_2PO_4, 1.0g yeast extract, 5.0g glucose, 5.0g glutamic acid, 0.2g $NaHCO_3$, 5ml PII trace metals, 2ml vitamin mix, and 2ml antibiotic mix. The culture was incubated at 30°C on a rotary shaker (200 rpm). After 2 days the culture was at a moderate density and actively growing. 20ml of this actively growing culture was used to inoculate an 1 liter fermenter containing 1000ml of the same culture media except the concentration of the glucose and glutamate had been increased to 40g/l (M20 media). The fermenter was maintain at 30°C and pH 7.4, with aeration at 1 vol/min, and mixing at 400 rpm. After 48 hr, the concentration of the cells in the fermenter was 18.5g/l. Aeration and mixing in the fermenter was turned off. Within 2-4 minutes, the cells flocculated and settled in the bottom 250 ml of the fermenter. This concentrated zone of cells had a cell concentration of 72g/l. This zone of cells can be siphoned from the fermenter, and: (1) transferred to another reactor for a period of nitrogen limitation (e.g., combining the highly concentrated production of several fermenters); or (2) harvested directly by centrifugation or filtration. By preconcentrating the cells in this manner, 60-80% less water has to be processed to recover the cells.

Example 12. Utilization of a variety of carbon and nitrogen sources

Fifty ml of M5 culture media in a 250ml erlenmeyer flask was inoculated with a colony of Schizochytrium sp. S31 (ATCC No. 20888) or Thraustochytrium sp. U42-2 (ATCC No. 20891) picked from an agar slant. The M5 media was described in Example 4 except for 2ml vitamin mix, and 2ml antibiotic mix. The culture was incubated at 30°C

on a rotary shaker (200 rpm). After 2 days the culture was at a moderate density and actively growing. This culture was used to inoculate flasks of M5 media with one of the following substituted for the glucose (at 5g/l): dextrin, sorbitol, fructose, lactose, maltose, sucrose, corn starch, wheat starch, potato starch, ground corn; or one of the following substituted for the glutamate (at 5g/l): gelysate, peptone, tryptone, casein, corn steep liquor, urea, nitrate, ammonium, whey, or corn gluten meal. The cultures were incubated for 48 hours on a rotary shaker (200 rpm, 27°C). The relative culture densities, representing growth on the different organic substrates, are illustrated in Tables 7-8.

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Table 7. Utilization of Nitrogen Sources

N-Source	Strains	
	Thraustochytrium sp. U42-2 ATCC No. 20891	Schizochytrium sp. S31 ATCC No. 20888
glutamate	+++	+++
gelysate	+++	+++
peptone	++	++
tryptone	++	++
casein	++	++
corn steep liquor	+++	+++
urea	+	++
nitrate	++	+++
ammonium	+	+++
whey	+++	+++
corn gluten meal	+++	+++

+++ = high growth
++ = medium growth
+ = low growth
0 = no growth

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Table 8. Utilization of Organic Carbon Sources.

C-Source	Strains	
	Thraustochytrium sp. U42-2 ATCC No. 20891	Schizochytrium sp. S31 ATCC No. 20888
glucose	+++	+++
dextrin	+++	+++
sorbitol	+	+
fructose	+	+++
lactose	+	+
maltose	+++	+
sucrose	+	+
corn starch	+++	+
wheat starch	+++	+
potato starch	+++	+
ground corn	+++	0

+++ = high growth
++ = medium growth
+ = low growth
0 = no growth

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Example 13. Feeding of thraustochytrid-based feed supplement to brine shrimp to increase their omega-3 HUFA content

Cellular biomass of Thraustochytrium sp. 12B (ATCC 20890) was produced in shake flasks in M-5 medium (see Example 6) at 25°C. Cellular biomass of Thraustochytrium sp. S31 (ATCC 20888) was produced in shake flasks in M-5/10 medium (see Example 9) at 27°C. The cells of each strain were harvested by centrifugation. The pellet was washed once with distilled water and recentrifuged to produce a 50% solids paste. The resulting paste was resuspended in sea water and then added to an adult brine shrimp culture as a feed supplement. The brine shrimp had previously been reared on agricultural waste products and as a result their omega-3 HUFA content was very low, only 1.3 - 2.3% of total fatty acids (wild-caught brine shrimp have an average omega-3 HUFA content of 6 - 8% total fatty acids). The brine shrimp (2 - 3/mL) were held in a 1 liter beaker filled with sea water and an airstone was utilized to aerate and mix the culture. After addition of the feed supplement, samples of the brine shrimp were periodically harvested, washed, and their fatty acid content determined by gas chromatography. The results are illustrated in Figs. 8 - 9. When fed the thraustochytrid-based feed supplement as a finishing feed, the omega-3 content of the brine shrimp can be raised to that of wild-type brine shrimp within 5 hours if fed strain 12B or within 11 hours when fed strain S31. The omega-3 HUFA content of the brine shrimp can be greatly enhanced over that of the wild type if fed these feed supplements for up to 24 hours. Additionally, these feed supplements greatly increase the DHA content of the brine shrimp, which is generally only reported in trace levels in wild-caught brine shrimp.

Example 14. Feeding of thraustochytrid-based feed supplement to laying hens to produce omega-3 HUFA enriched eggs

Cellular biomass of Thraustochytrium sp. S31 (ATCC 20888) was produced in a 10 liter fermenter in M-5/10 medium (see Example 9) at 27°C. The cells of Thraustochytrium sp. S31 (ATCC 20888) were harvested by centrifugation, washed once with distilled water and recentrifuged to produce a 50% solids paste. This cell paste was then treated in one of two ways: 1) lyophilized; or 2) mixed with ground corn to produce a 70% solids paste and then extruded at 90 - 120°C and air dried. The resulting dried products were then ground, analyzed for omega-3 HUFA content, and mixed into layers rations at a level to provide 400 mg of omega-3 HUFA per day to the laying hens (400 mg omega-3 HUFA/100 grams layers ration). The resulting eggs were sampled over a period of approximately 45 days and analyzed by gas chromatography for omega-3 HUFA's. Eggs with up to 200 - 425 mg omega-3 HUFA's/egg were produced by the hen fed omega-3 supplement. When cooked, these eggs did not exhibit any fishy odors. The control hens produced eggs with only approximately 20 mg omega-3 HUFA/egg. There was no significant difference between the number of eggs laid by the control group and the hen fed the omega-3 supplement. There was also no difference in the color of yolks of the eggs produced with the feed supplement and the control diet.

Example 15. Production of high purity (>90% purity omega-3 HUFA or >90% purity HUFA fatty acids mixtures)

Cellular biomass of Thraustochytrium sp. S31 (ATCC 20888) was produced in a 10 liter fermenter in M-5/10 medium (see Example 9) at 27°C. The cells of this strain were harvested by centrifugation. Approximately 5 g of the cell paste was placed in the 350 mL stainless steel grinding chamber of a Bead-Beater bead mill which was filled 1/2 way with 0.5 mm glass beads. The

remaining volumes of the chamber was filled with reagent grade MeOH and the cells homogenized for two 3 minute periods. During the bead mill operation, the stainless steel chamber was kept cold by an attached ice bath. The solution of broken cells was poured into a flask to which was added both chloroform and a 2M NaCl solution in water to bring the final solution to approximately 1:1:0.9 (chloroform:MeOH:water). The solution was then poured into a separatory funnel and shaken several times to help move the lipids into the chloroform fraction. After the solution was allowed to settle for several minutes, the chloroform fraction was collected into a flask, another portion of fresh chloroform added to the separatory funnel and the extraction repeated. This fraction of chloroform was then collected from the separatory funnel and the two chloroform portions combined. The chloroform was then removed (and recovered) by using a roto-vap rotary vacuum evaporation device operated at 40°C. A portion (300mg) of the remaining lipids was removed and hydrolyzed for 6 hours at 60°C (under nitrogen gas) in 50 mL of solution of methanolic NaOH (10 mL of 0.3 N NaOH diluted to 100mL with MeOH) in a 150 mL teflon lined screw capped bottle. The nonsaponifiable materials (sterols, hydrocarbons, etc.) were then removed by phase separating the solution with two 50 mL portions of petroleum either in a separatory funnel, discarding the ether fraction each time. The remaining solution was then acidified by addition of 3 mL of 6 N HCl and the free fatty acids extracted with two 50 mL portions of petroleum ether. Five mL portion of the ether solution containing the free fatty acids was placed in three 13mm X 100mm test tubes and the ether removed by blowing down the solution under a flow of nitrogen gas. Two mL portions of either petroleum ether, hexane or acetone were then added to one of tubes, which was then caped and placed in a solution of dry ice and ethanol (-72 to -74°C) to allow the non-HUFA fatty acids to crystallize. When

crystallization appeared complete, the culture tubes were placed in 50 mL polycarbonate centrifuge tubes that had been filled with finely powdered dry ice. These tubes were then placed in a refrigerated centrifuge at -10°C and centrifuged for 3-5 minutes to 10,000 rpm. The supernatant was then quickly removed from each tube with a pasteur pipet and placed in a clean culture tube. The solvent was removed from the supernatants by blowing down under N_2. The fatty acids were then methylated in methanolic H_2SO_4 (4 mL H_2SO_4 in 96 mL MeoH) at 100°C for 1 hr in teflon lined, screw capped tubes under N_2. The fatty acid methyl esters were then quantified by gas chromatography (HP 5890 gas chromatograph, Supelco SP 2330 column; column temp = 200°C; detector and injector temp = 250°C; carrier gas = nitrogen). The composition of the fatty acid mixtures obtained were: (ether) 93.1% HUFA's - 23.4% C22:5n-6 + 69.7% 22:6n-3; (hexane) 91.5% HUFA's - 66.8% 22:6n-3 + 22.1% 22:5n-6 + 2.6% 20:5n-3; (acetone) 90.0% HUFA's - 65.6% 22:6n-3 + 21.8n-6 + 2.6% 20:5n-3.

A fatty acid mixture containing >90% omega-3 HUFA's can be obtained by running the above process on harvested biomass of a strain of thraustochytrid such as 12B (ATCC 20890).

General Concluding Remarks

The following novel strains, isolated according to the method of the invention, were placed on deposit at the American Type Culture Collection (ATCC), Rockville, MD, as exemplars of the organisms disclosed and claimed herein.

Strain	ATCC No.	Deposit Date
Schizochytrium S31	20888	8/8/88
Schizochytrium S8	20889	8/8/88
Schizochytrium 12B	20890	8/8/88
Thraustochytrium U42-2	20891	8/8/88
Schizochytrium 23B	20892	8/8/88

The present invention, while disclosed in terms of specific organism strains, is intended to include all such methods and strains obtainable and useful according to the teachings disclosed herein, including all such substitutions, modifications and optimizations as would be available expedients to those of ordinary skill in the art.

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